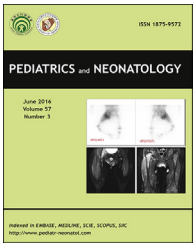
Pediatrics and Neonatology (2016) 57, 174–180

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.pediatr-neonatol.com



ORIGINAL ARTICLE

Urinary Transforming Growth Factor β-1 as a Marker of Renal Dysfunction in Sickle Cell Disease

Emad E. Ghobrial [a,*], Hanan A. Abdel-Aziz [a], Ahmed M. Kaddah [a], Nesma A. Mubarak [b]

[a] *Department of Pediatrics, Faculty of Medicine-Cairo University, Cairo, Egypt*
[b] *Ministry of Health, Cairo, Egypt*

Received Nov 10, 2014; received in revised form Feb 17, 2015; accepted May 12, 2015
Available online 24 October 2015

Key Words
biomarkers;
sickle cell nephropathy;
transforming growth factor β-1

Background: Sickle cell disease (SCD) is a genetic disorder that results in deformity of red blood cells. Renal dysfunction affects 5–18% of patients with SCD. To date, few studies have described urinary levels of transforming growth factor β-1 (TGF-β1), which is a marker of fibrosis, as a biomarker in identifying patients at risk of developing renal disease in SCD. The aim of this study is to determine prevalence of sickle cell nephropathy in Egyptian SCD patients. We aimed also to evaluate the association of urinary TGF-β1 with other conventional biomarkers of renal damage in SCD patients to identify a novel renal biomarker for early diagnosis of sickle nephropathy.

Methods: We examined 40 SCD patients, 21 with sickle cell anemia, 16 sickle thalassemia, and three with sickle trait. We compared them to 20 control children with matched age and sex. The study was held in the time period between May 2013 and December 2013 in the Hematology Clinic, New Cairo University Children Hospital, Cairo, Egypt.

Results: Urinary excretion of TGF-β1 was 7.07 ± 1.91 ng/mL in SCD patients versus 2.23 ± 0.76 ng/mL in control children ($p < 0.001$). SCD patients had elevated estimated glomerular filtration rate (177.44 ± 35.6 mL/min/1.73 m^2), denoting a state of glomerular hyperfiltration. 47.5% of SCD patients had microalbuminuria. Urinary TGF-β1 correlated positively with microalbuminuria and estimated glomerular filtration rate ($p = 0.001$ and $p = 0.018$, respectively).

* Corresponding author. 72nd Mohamed Abdel-Moneam Street, off Rayel Street, Helwan, Cairo, Egypt.
E-mail address: dr.ememadmil@yahoo.com (E.E. Ghobrial).

http://dx.doi.org/10.1016/j.pedneo.2015.05.001

Conclusion: We concluded that urinary TGF-β1 may serve as a marker of early renal injury in SCD.

1. Introduction

Sickle-cell disease (SCD) is a multisystem disease, associated with episodes of acute illness and progressive organ damage and it is one of the most common severe monogenic disorders worldwide.[1] Sickle nephropathy (SN) is one of the most serious complications of SCD. It is characterized by insufficient angiogenesis due to the imbalance between proangiogenic and antiangiogenic process. As the disease progresses, end-stage renal disease develops.[2]

Patients with SCD must be considered at risk for chronic kidney disease (CKD), and they should have regular (at least once per year) measurements of the urinary albumin to creatinine ratio for early detection in children. Prolonged hyperfiltration results in renal damage and the development of proteinuria. The hyperfiltration and proteinuria cause glomerulosclerosis and reduction in kidney function.[3]

Proteinuria begins as microalbuminuria (MA) and progresses to overt proteinuria as the kidney sustains further damage resulting from SCD. MA is one of the earliest markers of kidney damage and has been found to be directly related to age and inversely related to hemoglobin levels among SCD children.[4]

Angiotensin II also stimulates growth factors, including transforming growth factor β-1 (TGF-β1), a potent fibrogenic growth factor that may play a significant role in pathogenesis of SN.[5] It is a peptide of low molecular weight and has pleiotropic action. In the kidneys, it stimulates fibrogenesis through enhanced production of extracellular matrix proteins and nephron loss by various mechanisms, such as apoptosis of endothelial cells and podocytes.[6]

Whether the urinary levels of TGF-β1 has a diagnostic significance in the early prediction of SN in children with SCD is still to be determined.[7]

The aim of this study is to determine the prevalence of SN in Egyptian SCD patients and to determine the risk factors for SN. We aim also to evaluate the association of urinary TGF-β1 with other conventional biomarkers of renal damage in SCD patients [MA, serum creatinine, and estimated glomerular filtration rate (eGFR)] in order to identify a novel renal biomarker for early diagnosis of SN.

2. Methods

This is a cross-sectional study conducted on a cohort of 40 SCD patients who attended the Hematology Clinic, New Cairo University Children Hospital (NCUCH), Cairo, Egypt during their regular health maintenance visits, between May 2013 and December 2013.

SCD patients aged 2—18 years diagnosed by hemoglobin electrophoresis were included in the study. Patients with clinical or laboratory findings of previous or current renal disease with any organ failure or with diseases that may cause elevation of urinary level of TGF-β1 (e.g., cardiac diseases, neoplasm) were excluded from the study.

Twenty-one patients were diagnosed as sickle cell anemia (52.5%), 16 as sickle-thalassemia (40%), and three as sickle cell trait (7.5%) formed the patient groups. Eleven were boys and the other 29 were girls.

Thirty-eight of the 40 patients were treated with hydroxyurea, while six patients were on iron chelating treatment.

Twenty children with matched age and sex were selected from those who attended the pediatric general outpatient clinics at NCUCH; 14 were boys and six were girls. They were enrolled as controls for urinary TGF-β1. They were subjected to the same exclusion criteria as the patients.

Informed consent was taken from parents of all children participating in the study.

All patients were subjected to a full history [including age, sex, consanguinity, duration of illness, blood transfusion, vaso-occlusive crises, frequency of hospital admission, history of infections, splenic status, urinary symptoms (polyuria, hematuria, etc.), drug therapy (chelation and hydroxyurea) and complications] and physical examination including body weight and its percentile, standing height and its percentile, body mass index (BMI), vital signs [blood pressure (BP) and its percentile, where BP levels $< 90^{th}$ percentile are considered normal, levels between 90^{th}—95^{th} percentiles are considered prehypertension, and levels $> 95^{th}$ percentiles are considered hypertension], pallor, jaundice, and abdominal examination to detect hepatomegaly and splenomegaly. Medical records were reviewed for demographic data and clinical events.

The following laboratory investigations were done: complete blood count [using Sysmex KX-21N]; liver function tests (alanine aminotransferase, aspartate amino transferase and serum albumin), kidney function tests(blood urea nitrogen and serum creatinine) and iron indices (serum iron and serum ferritin) [using Beckman Coulter CX9pro]; hemoglobin electrophoresis [using Hyrys_Sebia]; urinalysis (urine samples were centrifuged; dipsticks were used to detect sugar and albumin, then urine samples were microscopically examined) and 24 hours urinary proteins. The results of hepatitis B surface antigen and hepatitis C antibodies using enzyme-linked immunosorbent assays for all patients were recorded.

eGFR was calculated for all patients using the Schwartz formula.[8]

Urinary TGF-β1 was measured for all patients. Normal range is 2—12 ng/mL. The determination of urinary TGF-β1 was accomplished by using a commercially available kit, Abcam's TGF-β1 Human ELISA kit, in a fresh urine sample

given by every patient. The kit is designed for the quantitative measurement of human TGF-β1 in serum, cell culture supernatants and urine.[9]

2.1. Statistical analysis

The SPSS (version 19.0; SPSS Inc., Chicago, IL, USA) and Microsoft Excel programs were used to tabulate the results and represent them graphically. Quantitative variables were expressed as mean and standard error. Qualitative variables were expressed as count and percentage. The one-way analysis of variance was used to test the differences between groups. The Duncan multiple comparison test was used to test the significant differences between each pair of groups. The Chi-square test was used to compare the distributions between groups. The Pearson correlation coefficient test used to test the significant correlations between the quantitative parameters within each group. A p value < 0.05 is considered significant.[10]

3. Results

The 40 patients included 29 girls (72.5 %) and 11 boys (27.5%). Table 1 shows comparison between the three patient groups and control group regarding mean age, sex, and the disease duration of patient groups.

The mean age of patients at time of first presentation was 2.6 ± 2.53 years. All patients were diagnosed before the age of 10 years. All patients were negative for hepatitis B and C viruses.

The 20 controls were six girls (30%) and 14 boys (70%). Their mean age at the time of the study was 8.12 ± 4.16 years.

There were no statistically significant differences with regard to mean age and sex between the patient groups and the control group.

Table 2 shows comparison between three patient groups and control group for mean weight, mean height, and mean BMI, and indicates that there were no statistically significant differences in these.

There was a statistically significant difference between the different study groups regarding the systolic BP (SBP) and diastolic BP; however, the highest mean blood pressure was found in the sickle cell group, followed by sickle thalassemia group, and then the control and the sickle trait groups were nearly equal ($p < 0.001$). Although SBP was normal ($< 90^{th}$ percentile) in all study groups, 13 of 40 patients (32.5%) presented with diastolic prehypertension (90^{th}–95^{th} percentile), and four of 40 patients (10%) had diastolic hypertension.

Patients of the sickle cell group showed significantly higher level of adult hemoglobin (Hb) and sickle Hb than that in other groups of patients ($p = 0.036$ and $p = 0.001$ respectively), while patients of sickle thalassemia group showed significantly higher level of fetal Hb than other groups of patients ($p = 0.001$). Our study revealed that 95% of SCD patients participating in our study were on hydroxyurea treatment, 20% of whom had never received blood transfusion.

Table 3 shows a comparison between the four groups for complete blood count. The sickle trait group showed significantly higher levels of Hb, hematocrit, and red blood corpuscles, followed by the sickle cell anemia group then the sickle thalassemia group ($p = 0.001$ for all). Also mean corpuscular Hb and mean corpuscular Hb concentration showed significantly higher levels in the sickle trait group than other groups ($p = 0.007$ and $p = 0.001$, respectively). Red cell distribution width was significantly higher in the sickle thalassemia group, followed by the sickle cell anemia and then the sickle trait group ($p = 0.001$).

Serum albumin and BUN were significantly higher in the sickle trait group than in other patient groups ($p = 0.007$ and $p = 0.001$, respectively), while creatinine was

Table 1 Basic demographic data of all participants.

		Sickle cell ($n = 21$)	Sickle trait ($n = 3$)	Sickle thalassemia ($n = 16$)	Control ($n = 20$)
Age (y)	Mean ± SE	10.9 ± 5.4	3.83 ± 1	7.8 ± 4.6	8.12 ± 4.16
	<5 y 17 (28.3)	4 (19)	2 (66.67)	6 (37.5)	5 (25)
	5–10 y 21 (35)	6 (28.5)	1 (33.33)	4 (25)	10 (50)
	>10 y 22 (36.6)	11 (52.5)	0	6 (37.5)	5 (25)
Sex	Male 25 (41.66)	3 (14.3)	0	8 (50)	14 (70)
	Female 35 (58.33)	18 (85.7)	3 (100)	8 (50)	6 (30)
Disease duration	<5 y 19 (47.5)	9 (43)	3 (100)	7 (43.75)	
	5–10 y 11 (27.5)	4 (19)	0	7 (43.75)	
	>10 y 10 (25)	8 (38)	0	2 (12.5)	

Data are presented as n (%) unless otherwise indicated.
SE = standard error.

Table 2 Anthropometric measures.

	Sickle cell	Sickle trait	Sickle thalassemia	Control	F	*p*
Weight (kg)	34.07 ± 4	15.33 ± 1.3	26.06 ± 3.4	26.97 ± 2.9	1.892	0.141
Height (cm)	131.4 ± 5.5	102.3 ± 5.3	119.25 ± 7	124.7 ± 4.5	1.646	0.189
BMI (kg/m^2)	18.41 ± 0.8	14.62 ± 0.3	16.45 ± 0.7	16.28 ± 0.7	2.045	0.118

Data are presented as mean ± standard error.
BMI = body mass index.

Table 3 Complete blood and reticulocyte counts.

	Sickle cell (*n* = 21)	Sickle trait (*n* = 3)	S. thalassemia (*n* = 16)	Control (*n* = 20)	F	*p*
Rtx (%)	4.43 ± 0.51	4.9 ± 0.49	6 ± 0.82	*	1.493	0.238
Hb (g/dL)	9.10 ± 0.42	11 ± 0.51	8.88 ± 0.37	11.72 ± 0.21	15.210	0.001
RBCs 10^{12}/L	3.25 ± 0.14	3.71 ± 0.23	3.39 ± 0.16	4.47 ± 0.04	20.579	0.001
Hct (%)	26.36 ± 1.34	32.4 ± 1.77	26.06 ± 0.87	36.72 ± 0.62	24.273	0.001
MCV (fl)	81.87 ± 1.63	88.46 ± 1.86	82.11 ± 4.7	81.65 ± 1.31	0.328	0.805
MCH (pg)	28.54 ± 0.64	31.73 ± 0.84	26.48 ± 0.89	26.66 ± 0.43	4.503	0.007
MCHC (g %)	34.29 ± 0.29	35 ± 1	33.96 ± 0.57	31.87 ± 0.44	7.244	0.001
RDW (%)	18.61 ± 0.79	14.7 ± 0.95	18.85 ± 0.87	14.83 ± 0.35	8.064	0.001
PLT 10^9/L	368.4 ± 24.93	564.3 ± 5.54	245.6 ± 30.02	350.7 ± 21.84	8.860	0.001
TLC 10^9/L	9.16 ± 0.82	8.93 ± 0.4	8.4 ± 1.09	9.07 ± 0.59	0.157	0.925

Data are presented as mean ± standard error.
Hb = hemoglobin; Hct = hematocrit; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; Plt = platelet count; RBCs = red blood corpuscles; RDW = red cell distribution width; Rtx = reticulocyte count; SE = standard error; TLC = total leukocyte count.
* Reticulocytic counts were not done for control group.

significantly higher in the sickle thalassemia group ($p = 0.032$), taking in consideration that the highest creatinine level was 0.7, which is considered normal for age. The levels of BUN and creatinine were significantly lower in the overall SCD patients (BUN 8.5 ± 1.1 mg/dl, creatinine 0.4 ± 0.1 mg/dl), compared with the values in controls (BUN 12.1 ± 4.17 mg/dl, creatinine 0.5 ± 0 mg/dl). Regarding urine analysis, red blood corpuscles in urine were significantly higher in the sickle thalassemia group ($p = 0.001$).

Table 4 shows a comparison between the four groups for 24 hours urinary proteins, MA, eGFR, and urinary TGF-β1. MA was found in 19 of 40 SCD patients (47.5%) [12 had sickle cell anemia (57%) and 7 had sickle thalassemia (44%)], while none of the sickle trait patients presented with MA. By contrast, MA was present in four of the 20 control children (20%).

The mean value of eGFR was 177.44 ± 35.6 mL/min/1.73 m^2 and the mean value of urinary TGF-β1 was 7.07 ± 1.91 ng/mL. The highest level of eGFR and urinary TGF-β1 was found in the sickle cell anemia group followed by the sickle trait group then the sickle thalassemia group and then the control group which had the lowest values ($p = 0.007$ for eGFR and $p = 0.001$ for TGF-β1).

Table 4 Twenty-four-hour urinary protein, MA, eGFR, and urinary TGF-β1.

	Sickle cell (*n* = 21)	Sickle trait (*n* = 3)	Sickle thalassemia (*n* = 16)	Control (*n* = 20)	F	*p*
24-h urinary proteins (mg/24 h)	174.4 ± 13.6	87.66 ± 3.9	164.68 ± 11.6	136.17 ± 8.9	3.955*	0.013
MA (*n* = 23)	12 (57.1)	0	7 (43.7)	4 (20)		
eGFR mL/min/1.73 m^2	187.28 ± 5.3	170.32 ± 7.5	165.85 ± 11.6	147.29 ± 7.7	4.514†	0.007
TGF-β1 ng/mL	8.03 ± 0.4	6.35 ± 0.3	5.96 ± 0.3	2.23 ± 0.1	58.437†	0.001

Data are presented as *n* (%) or mean ± standard error.
eGFR = estimated glomerular filtration rate; MA = microalbuminuria; TGF-β1 = transforming growth factor β-1.
* Significant different at $p < 0.05$ between groups.
† Highly significant different at $p < 0.01$ between groups.

There was a significant positive correlation between eGFR and BMI in SCD patients (r = 0.34, $p = 0.027$), and eGFR had a significant negative correlation with serum creatinine level (r = −0.361, $p = 0.022$).

Table 5 shows correlation of TGF-β1 with age, BMI, Hb, fetal Hb, sickle Hb, BUN, serum creatinine, MA, and eGFR in the different patient groups. Urinary TGF-β1 tends to be higher at younger age. Urinary TGF-β1 had a strongly positive significant correlation with eGFR and MA. This correlation was found in the total of all SCD patients (r = 0.50 and r = 0.21; $p = 0.001$ and $p = 0.018$, respectively). However, TGF-β1 was significantly negatively correlated to Hb (r = −0.52, $p = 0.031$) and serum creatinine level (r = −0.45, $p = 0.018$) in the same two groups. A statistically significant negative correlation was found between urinary TGF-β1 and fetal Hb (r = 0.41, $p = 0.043$) only in patients with sickle cell anemia but not in the other groups.

4. Discussion

Standard renal function tests such as serum creatinine and eGFR become abnormal only when renal damage has become extensive and irreversible. Therefore, early markers are needed for early diagnosis of SN before kidney damage becomes irreversible, so that therapeutic interventions are effective at preventing progression of renal damage.[11]

Currently, MA and creatinine are the only markers of advanced renal injury. To find noninvasive urinary markers of early renal damage in SCD, we examined urinary levels of TGF-β1, which is a known profibrogenic cytokine that plays an important role in the progression of CKD. TGF-β1 has been shown to directly induce both increased synthesis and reduced degradation of matrix proteins, leading to a net accumulation of pathological matrix, thereby playing a role in the fibrotic process.[12]

The female predominance in the patient groups is explained by most older male adolescents being referred to the adult hematology clinic. The male predominance in the control group is explained by the control sample being taken randomly from those who attended the general pediatric outpatient clinics, NCUCH.

Our study revealed that there was no statistically significant difference in weight, height, and BMI between different groups of patients and controls. These results are consistent with that of Mohtat et al,[7] who did not find a significant difference in mean BMI between SCD patients and controls, but this was not consistent with Barden et al,[13] who concluded that children with SCD had impaired growth, delayed puberty and poor nutritional status.

Interestingly, our study identified normal SBP in all patients but elevated diastolic BP in 17 of 40 SCD patients (42.5%; diastolic hypertension in 10% and prehypertension in 32.5%). Aygun et al[14] identified no hypertensive patients despite abnormal calculated and measured GFR. In addition, in a Saudi Arabian cohort of children with SCD aged 1−16 years, BP measurements were within normal range.[15] However, in a study performed by Bodas et al[16] on SCD patients aged 3−18 years, 16.6% had elevated BP (hypertension in 8.3%, prehypertension in 8.3%).

The levels of BUN and creatinine were significantly lower in the overall SCD patients, compared with the values in controls, but both values were still within the normal range ($p = 0.001$ and $p = 0.032$ respectively). Our results also matched those of Thompson et al,[17] who found that serum creatinine levels were lower in patients with sickle cell disease compared with controls.

The low level of serum creatinine may be explained by the likelihood of tubular dysfunction in SCD patients, as well as an increased tubular secretion of creatinine.[18] In addition, diet influences the BUN and creatinine level.[19] Since diet and muscle mass were not controlled during our study, further investigations are necessary to study the possible contribution of these variables on the level of biochemical parameters.

In our study, the overall prevalence of MA in patients with SCD was 47%; the majority of them had sickle cell anemia (63.2%) and the rest had sickle thalassemia (36.8%), while no of sickle trait patients had MA.

A lower percentage was reported by McBurney et al,[4] who found MA in 19% of patients with SCD. Also, Datta

Table 5 Correlations of urinary transforming growth factor β-1 (TGF-β1).

	Urinary TGF-β1 correlations											
	All SCD patients ($n = 40$)			SCA ($n = 21$)			S trait ($n = 3$)			S thalassemia ($n = 16$)		
	r	p	Sig	r	p	Sig	r	p	Sign.	r	p	Sig
Age	−0.160	0.791	NS	−0.172	0.456	NS	−0.17	0.891	NS	−0.139	0.608	NS
BMI	0.206	0.203	NS	0.125	0.590	NS	0.498	0.668	NS	−0.086	0.750	NS
Hb	−0.783	0.045	S	−0.526	0.031	S	−0.68	0.523	NS	−0.114	0.673	NS
Hb F	−0.110	0.501	NS	−0.413	0.043	S	0.6	0.521	NS	−0.123	0.649	S
Hb S	0.109	0.502	NS	−0.413	0.063	NS	−0.57	0.608	NS	0.064	0.813	NS
BUN	−0.076	0.643	NS	0.032	0.889	NS	0.071	0.955	NS	0.395	0.130	NS
Creat	−0.45	0.003	S	−0.510	0.018	S	0.071	0.955	NS	−0.109	0.689	NS
MA	0.50	0.001	S	0.650	0.001	S	0.974	0.147	NS	0.257	0.336	NS
eGFR	0.213	0.018	S	0.042	0.030	S	−0.20	0.867	NS	0.075	0.784	NS

BMI = body mass index; BUN = blood urea nitrogen; Creat = creatinine; eGFR = estimated glomerular filtration rate; Hb = hemoglobin; HbF = fetal hemoglobin; HbS = sickle hemoglobin; MA = micro-albuminuria; NS = non-significant; S = significant.

et al[20] reported a 19.2% prevalence of MA among Indian children with SCD. Similarly Alvarez et al[21] recorded a prevalence of 16.8% among SS children and 18% amongst the SC group. Imuetinyan et al[15] found MA in 20.3%. The slight difference between the overall prevalence of MA and that in our study is probably due to the increased number of older SCD patients in our study (42.5% aged 10–18 years), as the prevalence of MA increases with increasing age as proved by a previous study done by King et al[22] on Jamaican children with SCD.

Our results demonstrate the presence of higher eGFR in SCD patients than in controls denoting hyperfiltration ($p = 0.007$). Similarly, in a study by Bodas et al,[16] on a cohort of SCD patients with mean age 12 ± 3 years, 73% of patients had elevated eGFR with mean value 140 ± 34.9 mL/min/1.73 m^2. Aygun et al[14] studied 85 children with SCD with mean age 9.5 ± 4.8 years, 77 with sickle cell anemia and eight with sickle–thalassemia, and revealed that 76% of patients had elevated eGFR with mean value 154 ± 37 mL/min/1.73 m^2. Also, Thompson et al[17] determined that eGFR was 19% greater in SCD patients than controls.

In contrast to our findings, a cross-sectional study by Yee et al[23] reported an 11.6% prevalence of eGFR < 90 mL/min/1.73 m^2 in their patients (mean age 11.4 ± 4.5 years), supporting the idea that CKD may be more common in the pediatric population.

In this study we found a significantly positive correlation between eGFR and BMI in SCD patients ($r = 0.34$, $p = 0.027$). Similarly, Lo et al[24] reported a significant interaction between eGFR and BMI ($p < 0.001$). This correlation may be explained by the fact that the Schwartz formula that was used to estimate the GFR depends on the height of the patients as a variable in the equation.[8]

We found a significantly negative correlation between eGFR and hemoglobin F only in the group of sickle cell anemia patients (Hb SS; $r = -0.139$, $p = 0.049$), which is consistent with results of Haymann el al[25] in a study on adult patients with homozygous SS hemoglobinopathy. They found that hyperfiltration status was significantly associated with lower hemoglobin and lower hemoglobin F.

In our study, urinary excretion of TGF-β1 was 7.07 ± 1.91 ng/mL in SCD patients and 2.23 ± 0.76 ng/mL in the control group ($p = 0.001$). The highest level was in sickle cell anemia patients, followed by sickle trait and then sickle thalassemia patients. Similarly Mohtat et al,[7] in a study on 51 SCD patients aged 2–21 years (42 HbSS, 8 HbSC, and 1 HbSD), found that urinary TGF-β1 was significantly higher in patients than in controls.

In this study, TGF-β1 tended to be higher at younger ages. Okamoto et al[26] also reported that the serum TGF-β1 level in children (age 0–14 years) was significantly higher than that of patients older than 15 years.

In addition, we observed that urinary TGF-β1 had a significantly negative correlation with Hb and fetal Hb levels in patients with sickle cell anemia (Hb SS; $r = -0.52$ and $r = 0.41$; $p = 0.031$ and $p = 0.043$, respectively). This result is consistent with that of Mohtat et al,[7] who found that patient with Hb < 9 g/dL had higher urinary TGF-β1 than patients with milder anemia. This may be explained by the fact that lower Hb levels (anemia) lead to tissue hypoxia which in turn worsens the renal condition on top of the renal pathology caused by the sickle cell disease itself.

Furthermore, we detected a significantly negative correlation between urinary TGF-β1 and serum creatinine ($r = -45$, $p = 0.018$), which may be explained by the fact that glomerular hyperfiltration contributed to lowering the levels of serum creatinine in SCD patients.[17]

Nevertheless, we found a strong positive correlation between urinary TGF-β1 and urinary proteins and eGFR in all groups of SCD patients studied ($r = 0.50$ and $r = 0.21$; $p = 0.001$ and $p = 0.018$, respectively) which are considered to be markers for the degree and severity of SN. This is in agreement with Mohtat et al,[7] who concluded that urinary TGF-β1 was a marker of early renal injury in SCD more than MA.

One major limitation of this study was small number of the study population.

In conclusion, urinary TGF-β1 could be used as a simple and sensitive test in the diagnosis of early renal dysfunction in patients with SCD as it correlated positively with eGFR and MA, which are considered to be the earliest markers of SN. Close follow up of SCD patients is important to identify patients at risk of developing SN. Also, it is of a great importance to take into consideration that lower hemoglobin levels are associated with higher risk of developing renal disease.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

We thank all patients and their parents and nurses in the hematology clinic. Also we thank the technicians in the Chemical Pathology department.

References

1. Weatherall D, Hofman K, Rodgers G, Ruffin J, Hrynkow S. A case for developing North-South partnerships for research in sickle cell disease. *Blood* 2005;**105**:921–3.
2. Carmeliet P. Angiogenesis in life, disease and medicine. *Nature* 2005;**438**:932–6.
3. McKie KT, Hanevold CD, Hernandez C, Waller JL, Ortiz L, McKie KM. Prevalence, prevention, and treatment of microalbuminuria and proteinuria in children with sickle cell disease. *J Pediatr Hematol Oncol* 2007;**29**:140–4.
4. McBurney PG, Hanevold CD, Hernandez CM, Waller JL, McKie KM. Risk factors for microalbuminuria in children with sickle cell anemia. *J Pediatr Hematol Oncol* 2002;**24**:473–7.
5. Wolf G. Renal injury due to renin-angiotensin-aldosterone system activation of the transforming growth factor-beta pathway. *Kidney Int* 2006;**70**:1914–9.
6. Blumenberg M, Gao S, Dickman K, Grollman AP, Bottinger EP, Zavadil J. Chromatin structure regulation in transforming growth factor-beta-directed epithelial-mesenchymal transition. *Cells Tissues Organs* 2007;**185**:162–74.
7. Mohtat D, Thomas R, Du Z, Boakye Y, Moulton T, Driscoll C, et al. Urinary transforming growth factor beta-1 as a marker of

renal dysfunction in sickle cell disease. *Pediatr Nephrol* 2011; **26**:275–80.
8. Schwartz GJ, Haycock GB, Edelmann Jr CM, Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. *Pediatrics* 1976;**58**: 259–63.
9. Wakefield LM, Letterio JJ, Chen T, Danielpour D, Allison RS, Pai LH, et al. Transforming growth factor-beta1 circulates in normal human plasma and is unchanged in advanced metastatic breast cancer. *Clin Cancer Res* 1995;**1**:129–36.
10. Bolton S, Bon C. *Pharmaceutical statistics: practical and clinical applications*. 5th ed. Boca Raton: Taylor and Francis; 2009.
11. Sundaram N, Bennett M, Wilhelm J, Kim MO, Atweh G, Devarajan P, et al. Biomarkers for early detection of sickle nephropathy. *Am J Hematol* 2011;**86**:559–66.
12. Gordon KJ, Blobe GC. Role of transforming growth factor-beta superfamily signaling pathways in human disease. *Biochim Biophys Acta* 2008;**1782**:197–228.
13. Barden EM, Kawchak DA, Ohene-Frempong K, Stallings VA, Zemel BS. Body composition in children with sickle cell disease. *Am J Clin Nutr* 2002;**76**:218–25.
14. Aygun B, Mortier NA, Smeltzer MP, Hankins JS, Ware RE. Glomerular hyperfiltration and albuminuria in children with sickle cell anemia. *Pediatr Nephrol* 2011;**26**:1285–90.
15. Imuetinyan BA, Okoeguale MI, Egberue GO. Microalbuminuria in children with sickle cell anemia. *Saudi J Kidney Dis Transpl* 2011;**22**:733–8.
16. Bodas P, Huang A, O'Riordan MA, Sedor JR, Dell KM. The prevalence of hypertension and abnormal kidney function in children with sickle cell disease: a cross sectional review. *BMC Nephrol* 2013;**14**:237.
17. Thompson J, Reid M, Hambleton I, Serjeant GR. Albuminuria and renal function in homozygous sickle cell disease: observations from a cohort study. *Arch Intern Med* 2007;**167**: 701–8.
18. Asnani MR, Lynch O, Reid ME. Determining glomerular filtration rate in homozygous sickle cell disease: utility of serum creatinine based estimating equations. *PLoS ONE* 2013;**8**:e69922.
19. Lima CS, Bottini PV, Garlipp CR, Santos AO, Costa FF, Saad ST. Accuracy of the urinary albumin to creatinine ratio as a predictor of albuminuria in adults with sickle cell disease. *J Clin Pathol* 2002;**55**:973–5.
20. Datta V, Ayengar JR, Karpate S, Chaturvedi P. Microalbuminuria is a predictor of early glomerular injury in children with sickle cell disease. *Indian J Pediatr* 2003;**70**:307–9.
21. Alvarez O, Montane B, Lopez G, Wilkinson J, Miller T. Early blood transfusions protect against microalbuminuria in children with sickle cell disease. *Pediatr Blood Cancer* 2006;**47**:71–6.
22. King L, MooSang M, Miller M, Reid M. Prevalence and predictors of microalbuminuria in Jamaican children with sickle cell disease. *Arch Dis Child* 2011;**96**:1135–9.
23. McPherson Yee M, Jabbar SF, Osunkwo I, Clement L, Lane PA, Eckman JR, et al. Chronic kidney disease and albuminuria in children with sickle cell disease. *Clin J Am Soc Nephrol* 2011;**6**: 2628–33.
24. Lo JC, Go AS, Chandra M, Fan D, Kaysen GA. GFR, body mass index, and low high-density lipoprotein concentration in adults with and without CKD. *Am J Kidney Dis* 2007;**50**:552–8.
25. Haymann JP, Stankovic K, Levy P, Avellino V, Tharaux PL, Letavernier E, et al. Glomerular hyperfiltration in adult sickle cell anemia: a frequent hemolysis associated feature. *Clin J Am Soc Nephrol* 2010;**5**:756–61.
26. Okamoto Y, Gotoh Y, Uemura O, Tanaka S, Ando T, Nishida M. Age-dependent decrease in serum transforming growth factor (TGF)-beta 1 in healthy Japanese individuals; population study of serum TGF-beta 1 level in Japanese. *Dis Markers* 2005;**21**: 71–4.